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Disturbance of approach-avoidance behaviors in non-human primates by stimulation of the limbic territories of basal ganglia and anterior insula.

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Abstract

The basal ganglia (BG) are involved in motivation and goal-directed behavior. Recent studies suggest that limbic territories of BG not only support reward seeking (appetitive approach) but also the encoding of aversive conditioned stimuli (CS) and the production of aversive-related behaviors (avoidance or escape). This study aimed to identify inside two BG nuclei, the Striatum and Pallidum, the territories involved in aversive behaviors and to compare the effects of stimulating these territories to those resulting from stimulation of the anterior Insula (aIns), a region that is well known to be involved in aversive encoding and associated behaviors. Two monkeys performed an approach/avoidance task in which they had to choose a behavior (approach or avoidance) in an appetitive (reward) or aversive (air-puff) context. During this task, either one (single-cue) or two (dual-cue) conditioned stimuli (CS) provided essential information about which context-adapted behavior should be selected. Microstimulation was applied during the CS presentation. Stimulation generally reduced approaches in the appetitive contexts and increased escape behaviors (premature responses) and/or passive avoidance (non-initiated action) in aversive context. These effects were more pronounced in ventral parts of all examined structures, with significant differences observed between stimulated structures. Thresholds to induce effects were lowest in the pallidum. Striatal stimulation led to the largest diversity of effects, with a sub-region even leading to enhanced active avoidance. Finally, aIns stimulations produced stronger effects in the dual-cue context. These results provide causal evidence that limbic territories of BG, like aIns, play crucial roles in the selection of context-motivated behaviors.

Introduction

The BG have long been implicated in goal-directed and reward-oriented behavior (Hollerman et al., 1998; Kawagoe et al., 1998; Hollerman et al., 2000; Tachibana and Hikosaka, 2012). Previous studies have shown that the ventral pallidum (VP) and the anterior striatum (STR) contain two populations of neurons that respond to positive and negative CS and/or to anticipation of unconditioned stimuli (US) with motivational valences that fit positive or aversive outcomes (Richard et al., 2014; Saga et al., 2017). Neuroimaging studies have identified specific limbic territories in the BG and cortical regions (e.g. the insula, orbitofrontal cortex, and anterior cingulate cortex) that respond to the anticipation and presentation of such positive and negative outcomes (O'Doherty et al., 2001; Jensen et al., 2003). Although single-unit recordings have established the general involvement of these areas in motivated behaviors (Kawagoe et al., 1998; Tachibana and Hikosaka, 2012), there is only little causal evidence that these territories contribute to decision-making processes that select behaviors (approach or avoidance) in fitting contexts. These decision processes are thought to rely crucially on appropriate evaluation of CS(s) acquired during instrumental conditioning (Rangel et al., 2008). Whether or not this function requires the BG and connected areas is an open question.

Anatomically, multiple sub-territories in the STR and pallidum (also named globus pallidus; GP) are involved in different circuits that link them to different cortical areas and other BG nuclei (Haber et al., 1990; Spooren et al., 1996; Francois et al., 2004; Calzavara et al., 2007). Specifically, the lateral ventral STR (Chikama et al., 1997; Sgambato-Faure et al., 2016) receives input from the aIns, which is thought to represent internal states by interoceptive perception (Craig, 2009). The ventro-median STR and the anterior caudate nucleus receive convergent inputs from dorsal and ventral frontal regions (Haber et al., 1990; Haber et al., 1995; Calzavara et al., 2007). These anterior

striatal territories project to the anterior GP including the VP (Spooren et al., 1996). GABAergic modulation and/or microstimulation applied to the VP (Grabli et al., 2004; Saga et al., 2017) and medial ventral STR induce anxiety-related behaviors, whereas the same perturbations in the lateral part of the ventral STR (Worbe et al., 2009; Worbe et al., 2011) produce an apathetic state with loss of food motivation. The specific roles of these STR sub-territories in these two motivational domains (aversive avoidance and food intake) remain to be elucidated. Furthermore, it has been shown that bicuculline injections inside the VP induced anxiety-related behaviors and influenced some limbic cortical areas such as the amygdala and aIns (Galineau et al., 2017). All these findings suggest that limbic cortico-BG loops are specifically involved in the control of aversive-related behaviors, such as escape and avoidance behaviors in aversive contexts.

The aim of this study was to determine the relative contributions of limbic cortico-BG territories to the context-dependent selection of value-based behaviors. Because CSs provide contextual values informing this process, and because we have previously shown CS encoding activity in the STR and the VP (Richard, 2014; Saga et al., 2017), we disturbed neuronal activity of these structures during CS presentation. The monkeys had learned the approach or avoidance in tasks with opposite USs (appetitive-aversive) based on specific CSs. We chose the electrical microstimulation approach because it offers two advantages over pharmacological stimulation: first, it can disturb transiently during a trial-specific period, and second, these disturbances can be repeated on spatially close territories of the same structure.

The stimulation disturbed value-based behavioral selection in a context-dependent manner, particularly when stimulation was applied in the ventral parts of all structures. We found two major abnormal behaviors that were rarely observed in the control sessions:

1) reductions of approach behaviors 2) increase of error rates (non-initiated action and premature response) in both positive and negative contexts, respectively. The different effects of stimulation inside these three studied structures suggest that each of these structures in the limbic cortico-BG circuit plays a distinct role in value-based decision processes and the control of context-adapted behaviors.

Materials and methods

The same animals as in a previous study (Saga et al., 2017) were used in this study: a female rhesus monkey (*Macaca mulatta*, 5 kg; Monkey T) and a male fascicularis monkey (*Macaca fascicularis*, 4 kg; Monkey C). Animal care and housing were in accordance with the National Institute of Health guidelines (1996) and the recommendations of the European Communities Council Directive of 2010 (2010/63/UE) and the French National Committee (87/848). The project received the ethical approval number (991-2015051213399473) by the national ethical committee.

Apparatus

During the experimental sessions, the monkeys sat in a chair with their head fixed. The experiments were conducted in a darkened room. A plastic bar with an infrared sensor was installed at waist level in front of the chair, which the monkeys could easily hold and release with their left hand. A 19-inch color video monitor equipped with a touch-sensitive screen was placed in front of the monkey. Eye movements, eye positions, and blinking were monitored at 120 Hz using an infrared eye-tracking system (resolution, 0.25° visual angle; DQW-1 version 1.20; ISCAN Inc., MA, USA). Licking was detected whenever the tongue interrupted an infrared beam installed in the juice delivery system. The behavioral data were collected at 1,000 Hz with a Spike2 data acquisition system (Cambridge Electronic Design Ltd., CB, England). Presentation (Neurobehavioral Systems, Inc., CA, USA) and Scenario manager (Institut des Sciences Cognitives Marc Jeannerod, Bron, France) software was used to control the behavioral task, along with solenoid valves that opened and closed the reward delivery system and the air-puff system. Both systems making only very weak and indistinct noises. Single drops of 0.2 ml of apple juice served as an appetitive unconditioned stimulus (appetitive US) and were delivered via a small plastic hole placed in front of the monkey's mouth. Single puffs of air delivered at 1.5-2.0 bar (25-

35 psi) served as an aversive outcome (aversive US). The air puffs were directed to the left side of the monkey's face, including the cheek and eye, and delivered through a tube with its opening set at a distance of 10–15 cm from the face.

Surgery

After the monkeys had learned the task, we implanted the head fixation system and a chamber to perform microstimulation in a specific structure and territories. Details of the surgical procedures are described in Worbe et al. (2011) and Galineau et al. (2017). Briefly, aseptic surgery was performed under gaseous anesthesia (Isoflurane 1%, nitrogen monoxide 50% and oxygen 50%) provided through intratracheal intubation. For each monkey, a magnetic resonance imaging (MRI) compatible delrin chamber (Unimecanique, Epinay-sur- Seine, France) was stereotaxically implanted. The skull under the chamber was removed but the dura mater was left intact. The recording chamber was positioned with respect to the anterior commissure (AC) coordinates obtained from T1-weighted MRI scan (1.5 T; CERMEP, Lyons, France). The centre of the chamber was aligned with the AC to give easy access to the anterior STR (AC +6 to +3), the anterior GP (AC 0 to −1) and anterior Ins (AC+3 to -2). Plastic and titanium screws were implanted in the skull to attached its with the chamber and the head fixation ring using acrylic resin. Finally, antibiotics and analgesics were used to prevent postsurgical infection and pain at least consecutive five days after surgery.

Behavioral tasks

In the current study, we used the same delayed-response tasks as in our previous investigation, comprising a dual-cue condition (Fig. 1A) and a single-cue condition (Fig. 1B) presented in alternating blocks (see below and Saga et al., 2017). The dual-cue condition enabled us to investigate value-based decision-making in a condition where CSs with opposite values are presented simultaneously, whereas the appetitive and aversive

single-cue condition allowed us to control the integrity of pure reward-seeking (approach behavior) and aversive-related behaviors (avoidance behaviors). Thus, the dual-cue condition implements competition between appetitive and aversive options, whereas the single-cue condition provides purely positive or negative motivational contexts.

To start a trial in either condition, the monkeys had to hold the bar with their left hand (Figs. 1A and B). A small white dot (starting point) immediately appeared at the center of the screen. After 1.3 s, it was replaced by two (dual-cue condition, Fig.1A) or one CS (single-cue condition, Fig.1B). CSs were presented for 1.0 s, pseudo-randomly placed on either the left or right side (single-cue condition) or on both sides (dual-cue condition) of the touch screen. The CSs provided appetitive or aversive contexts or competitive conditions; that is, they informed the monkeys that depending on their future response (see below), they would either obtain an appetitive outcome (apple juice) or an aversive outcome (air-puff) as USs, or avoid these USs. After the CSs had disappeared, a random delay period of 1.5–2.0 s occurred.

Next, green square targets (visual angle of 12°) were presented for a maximum of 2.0 s on the left and right sides of the screen. In both cuing conditions, the monkeys had to select one of the two targets by touching the screen. In the single-cue condition, selecting the target in the same position in which the CS had been presented corresponded to approach, whereas selecting the target in the other position corresponded to avoidance. The targets disappeared as soon as one of them had been selected. If the monkeys selected the target at the position where the CS had been shown, either the liquid reward or the air-puff was delivered after a random delay of 1.5–2.0 s. By contrast, if they selected the target at the opposite position, nothing happened. Thus, in this case the monkeys missed out on the opportunity to earn a reward or successfully prevented the air-puff. This entails that monkeys may have to select the same motor response in order to either approach the positive outcome (juice) or avoid the negative outcome (air-puff), depending on the

appetitive or aversive nature of the presented CS. It is only the characteristics of their behaviors (reaction time and movement time) that inform us if their behaviors are guided to obtain the reward or to avoid the aversive outcome. By contrast, in the dual-cue condition, the outcome associated with the CS was delivered according to the selected target position (Fig.1A). The monkeys therefore had the possibility to explicitly between choose between the positive and negative outcomes in the dual-cue condition

To maintain the motivation of the monkeys to perform in the single-cue condition, the aversiveness of the air-puff had to be limited. Aversive trials occurred only after appetitive trials, so that aversive trials were not repeated except in the case of error trials after ITI. In addition, the monkeys were never punished, even if they failed to complete a trial (i.e., error responses). Thus, in the single-cue condition, the monkeys had a chance to refuse to perform aversive single-cue trials, but they had to complete an aversive trial in order to move on an appetitive trial. We examined the electrical stimulation effect during the CS presentation with block-designed behavioral conditions (Fig. 1, yellow zone). A block consisted of 35 trials (for Monkey C) or 50 trials (for Monkey T) of the single-cue condition, then 30 trials (for Monkey C) or 40 trials (for Monkey T) of the dual-cue condition. In the single-cue condition, 60% (i.e., 21 or 30) of the trials were appetitive and 40% (i.e., 14 or 20) were aversive single-cue trials. The different numbers of trials contained in a block were chosen so as to optimize motivation.

Three different types of error could occur. First, trials in which monkeys released the bar prematurely before appearance of targets on the screen were categorized as premature responses. These responses could be interpreted as *impulsivity* (not being able to wait to give their choice in the goal to obtain reward) when they occurred before the appearance of the trigger (in appetitive trial during the first delay), or as *escape behavior* when facing an aversive CS or when predicting the risk of a negative outcome or an unpleasant event (peri-CS), as we have previously shown (Saga et al. 2017). Second, trials in which monkeys

produced no response at all during the 2-s target presentation were categorized as non-initiated actions. These errors can be a sign of indifference, a loss of motivation, or a behavioral response (i.e., freezing reaction or passive avoidance) to threat, excessive risk of a negative outcome, or to an unpleasant event. As we have shown previously, both premature responses and non-initiated actions are mainly expressed in the aversive context of this task and are increased during $GABA_A$ dysfunction inside the vSTR (Richard, 2014) and VP (Saga et al. 2017). After a premature response, the trial was stopped and the CS(s) disappeared immediately, followed by the ITI. The third error category consisted of trials in which monkeys touched outside of the target area, suggesting a visuo-motor disturbance.

To control for the possibility that outcomes or contexts were triggered by specific visual features of the CS rather than their learned motivational significance, we used different CS images that we associated with the same outcomes. In particular, we used five different sets of images including abstract object images, food images, and social images (monkey faces). Different CS images were presented in groups of 10 trials. The monkeys viewed all sets of images in each sub-block. All the sets of images were well learned by each monkey during several months of training before we initiated the stimulation period.

Physiological recordings and stimulation parameters

To localize target areas in each monkey, we acquired three high-resolution (0.6 x 0.6 x 0.6 mm^3) 3D T1-weighted MRI scans with a 1.5 T Sonata Siemens scanner. The MR images provided us with an estimate of the location and depth of the striatum, pallidum, and insula with respect to the cortical surface, and of the structures that the electrode would pass through before reaching each structure. We used a grid system that allowed us to access targeted structures in intervals of 1 mm. The pallidum was investigated at the anterior commissure (AC)+1 and AC0 where we previously studied the effect of bicuculline

injection (Fig.1E, (Saga et al., 2017)). The striatum was examined at AC+5 and AC+3 where previous studies found highly effective electrical stimulation sites (Fig.1F, (Worbe et al., 2011)). The insula was tested at the level from AC+3 to AC-2 (Fig.1G). Neuronal recordings were performed before to the stimulation to confirm the location of the stimulated sites inside the different studied nuclei. Neuronal activity was measured using epoxy-insulated tungsten microelectrodes (FHC Inc., ME, USA; resistance: 2–4 MΩ at 1 kHz) inserted into the brain through a 23-gauge guide tube that penetrated through the dura mater. A mechanical microdrive (NAN-A, Nan Instruments Ltd., Nazaret, Israel) was used to move the electrode in micrometer steps. The same microelectrodes were also used for the stimulations. Electrical stimulation was applied by a DAM WOI15 stimulator and was displayed on an oscilloscope. The stimulation was applied during the CS period with similar parameters to a previous study (Worbe et al., 2011), which had demonstrated that discontinuous long-train stimulation (700 ms) to the anterior STR induces disturbance of spontaneous behaviors. We explored effects with biphasic pulses (pulse duration 0.3 ms, frequency 200Hz), and the current intensity was between 0.1 and 0.6 mA (up to 0.8 mA for the insula) depending on the stimulated regions.

Stimulation was applied to the anterior part of the GP, STR, and insula (Figs.1E-G). To examine different locations inside each structure, the stimulation was tested every 0.5mm or 1mm with different current intensities. The stimulation was started at 0 mA (sham control stimulation) and subsequently, the intensity was increased from 0.1 mA to 0.6 mA in steps of 0.1 or 0.2 mA (for the pallidum and striatum), and from 0.2 to 0.8 mA for the insula. Sham stimulation was repeated after each change of stimulation site. A maximum of 2 to 3 sites were stimulated in each daily session and no more than 3 days of stimulation were carried out per week.

Data analysis

Behavioral analysis

For both the single-cue and dual-cue condition, we calculated behavioral outcomes such as reaction time (RT), movement time (MT), the proportion of complete trials, error trials, and approach and avoidance behaviors. The RTs were defined as the time between the appearance of the targets and releasing the response bar. The MTs were defined as the time between releasing the response bar and touching the target. A complete trial was registered if monkeys touched any target (irrespective of their choice, i.e., for either approach or avoidance in the single-cue condition, and choice of the appetitive or aversive outcome in the dual-cue condition). To detect stimulation effects on behavior, we analyzed the number of complete trials and the number of approach trials in the appetitive single-cue condition using two-way analysis of variance (ANOVA) with the following factors: stimulation territory and conditions (stimulation or control). We also performed a one-way ANOVA ($p < 0.01$, factor control vs. stimulation) to detect any significant effect either on the number of complete trials or the number of approach behaviors in the positive context (i.e. appetitive single-cue condition or dual-cue condition) compared to control sessions at the single stimulation site. To perform this, a block of stimulation sessions and control sessions were added to every set of three trials. Stimulation sites were considered as effective sites if they showed significant differences in these ANOVAs (effect on error response, approach behaviors in the positive context, or both); these stimulation sites were further analyzed regarding approach-avoidance behaviors and error response in each condition (i.e. premature response, non-initiated action) (Fig. 5-6). The number of premature responses and non-initiated actions were expressed as proportion of trials by session. For example, if the monkey performed 10 trials of the aversive single-cue condition and made six premature responses (60%), this site would be assigned more than 51% of premature responses (the highest intensity, Fig. 6).

Blinking and licking

To estimate how far in advance monkeys anticipated positive and negative outcomes, we calculated the average number of blinks and licks for the different motivational contexts in a given trial (Saga et al., 2017). To detect blinks, we used the vertical component of the eye movement trace (Matsumoto and Hikosaka, 2009). Specifically, we set a threshold and calculated downward movement of the eyelid during pre-CS presentation. We defined a blink as crossing the threshold within 1.0 s and calculated how many times the eyelid crossed the threshold. Blinking was analyzed for 1.0 s before US delivery because monkeys could estimate the time of the air-puff based on the time they touched the target although this period was randomized. To determine how far in advance monkeys anticipated the positive outcome (liquid reward), we measured predictive licking behavior. We counted the number of times the tongue interrupted the infrared sensor during the 1.0 s prior to US delivery.

Division of structures

In order to investigate stimulation effects for different territories in each structure, targeted structures were divided into the dorsal and ventral part. For the pallidum, this was done by the anatomical landmark of the AC, dividing the structure into dorsal (DP) and ventral (VP) at AC+1 and AC+0 (Fig.1E, see also Grabli et al., 2004). The striatum was divided at the line set boundary between the lateral part of the putamen and the medial part of the caudate across the bottom of the internal capsule, the ventral third of the anterior striatum (Fig.1F). Dorsal and ventral parts of the striatum are referred to as the dorsal striatum (dSTR) and the ventral striatum (vSTR). The insula was divided into the dorsal (dIns) and ventral part (vIns) at the middle of the insula (Fig.1G: red lines).

Results

Behavioral performance during the control sessions.

The monkeys performed both the single-cue condition with mixed positive and negative contexts and the dual-cue condition within a block design. They started to perform the conditions without electrical stimulation (sham control sessions) and showed a high percentage of completed trials for the appetitive single-cue and dual-cue conditions. The proportions of complete trials were 98.9 ± 2.0 and 99.6 ± 1.4% (mean ± SEM) in the dual-cue condition and 98.6 ± 3.6 and 99.3 ± 2.3% in the appetitive single-cue condition in Monkey T and Monkey C, respectively. For the aversive single-cue condition, the proportions of completed trials were 88.6 ± 1.2 and 96.9 ± 0.8% (Fig.1C left panel). The apparent difference in this index between conditions was significant (one-way ANOVA, $F_{2, 249} = 74.46$, $p = 1.11 \times 10^{-16}$, for Monkey T and $F_{2, 384} = 8.29$, $p = 0.0003$ for Monkey C). Both monkeys showed a significant difference in the number of completed trials between the aversive and the appetitive condition ($p < 0.01$ for both monkeys, post-hoc Turkey's HSD test) and between the aversive and dual-cue condition ($p < 0.01$ for both monkeys), whereas no such difference was found between the appetitive and dual-cue condition ($p > 0.1$ post-hoc Turkey's HSD for both monkeys), Thus, both monkeys appeared to show higher motivation to perform the dual-cue condition and the appetitive single-cue condition than the aversive single-cue condition. Both monkeys approached the target associated with the appetitive US in more than 95% of trials of the dual-cue condition and the appetitive single-cue condition (Fig.1C middle panel). On the other hand, they actively avoided the air-puff in the aversive single-cue context, albeit with lower probability than that associated with the appetitive behavior in the appetitive context (Fig.1C middle panel, 63.9 ± 1.1% in Monkey T and 55.2 ± 0.9% in Monkey C). One may wonder whether the monkeys had not learned the association of the CSs with the USs in that condition. However, our previous study shows that these two monkeys had similarly learned the positive and negative contexts provided by CSs (Saga et al., 2017). Moreover, blinking and

licking behaviors clearly showed differences during the anticipation period between the aversive and appetitive motivational contexts (Fig. 1D). The fact that no external feedback was given when the monkeys succeeded in avoiding the air-puff in the aversive single-cue condition might explain the lower proportions of avoidance for aversive US. Moreover, the fact that avoidance is linked naturally to omissions or no-go responses (Guitart-Masip et al., 2012) could lead to a difficulty in maintaining conditioned avoidance even for well-associated CSs with USs (Seymour et al., 2015). Consistently, we also found that the RTs were slower in the aversive single-cue condition than in the dual-cue and appetitive single-cue condition (Fig.2B and C, "Cont", more than 300 tested trials in each trial type, $F_{2, 1909} = 389.91$, $p = 1.0 \times 10^{-10}$ for monkey T, $F_{2, 2109} = 369.85$, $p = 2.0 \times 10^{-10}$ for monkey C, one-way ANOVA post-hoc HSD test), suggesting that monkeys were discriminating between the differential motivational contexts provided by the appetitive and aversive CSs. Thus, they discriminated between different CSs based on their values and the CSs guided decisions to select the context-adapted behaviors (approach or avoidance).

Behavioral effects induced during stimulation sessions.

The stimulation was applied at 260, 87, and 95 sites in the anterior part of the STR, GP, and insula, respectively, including the dorsal and ventral parts. More than 45% of these stimulation sites showed effects (Table 1). The current intensity of stimulation had to be higher than 0.6mA to induce effects in the aIns on average (Table 1). In contrast, in the STR and GP, the intensity required to induce effects was considerably smaller (Table 1). For all studied structures, a higher intensity was required to induce effects for stimulation of the dorsal part compared to the ventral part. We compared the quantity of errors and approach behaviors for different intensities of current (i.e., 0.2mA versus 0.4mA etc.). However, there were no significant differences in terms of the number of errors or approach behaviors between different levels of current intensity except for DP (one-way ANOVA with factor of current intensity, $p = 0.0455$ for DP, $F_{4, 139} = 1.4211$, $F_{1, 115} = 0.1056$,

$p = 0.7457$ for dSTR, $F_{2, 384} = 1.3798$, $p = 0.2529$ for vSTR, $F_{2, 147} = 3.1553$, $p = 0.2302$ for VP, $F_{2, 39} = 0.2725$, $p = 0.7629$ for dIns, $F_{2, 75} = 2.2787$, $p = 0.0711$ for dIns).

Most of the effective sites showed disadvantageous effects, i.e., either a reduction of appetitive approaches or an increase of error responses, or both (Table 1 and Fig. 2). Figure 2A depicts the increase in error responses induced by each stimulation site in comparison to the control sham sessions. A one-way ANOVA showed a significant difference between stimulation sessions ($F_{6, 538} = 14.73$ in the dual-cue condition, $p = 1.11 \times 10^{-16}$, $F_{6, 538} = 17.87$ in the appetitive single-cue condition, $p = 1.11 \times 10^{-16}$, $F_{6, 538} = 38.94$, $p = 1.33 \times 10^{-15}$ in the aversive single-cue condition). As shown in figure 2A, stimulation induced error responses especially in the VP, compared to the other stimulation sites, in all conditions (Fig.2A, post-hoc Turkey's HSD test, $p < 0.05$). The vSTR stimulation induced error responses significantly in the aversive single-cue condition (Fig. 2A right) and a non-significant difference compared to the VP (post-hoc Turkey's HSD test, $p = 0.62$). Thus, error responses during stimulation sessions were widely observed, and the two BG territories (VP and vSTR) emerged as the most effective sites, especially in the aversive single-cue condition. However, the proportion of approach behaviors could be reduced due to either an increase of error responses or an increase of avoidance behavior. To clarify this, we checked the proportion of avoidance choices on non-error trials, i.e., when monkeys reached for the screen. Both monkeys showed increased numbers of avoidance choices for stimulation of the vSTR and VP, in both dual-cue and appetitive single-cue condition (Fig.2 D and E, $p < 0.05$ compared to control, two-tailed t-test). Monkey T showed further increases of avoidance choices during stimulation of the ventral insula and dorsal striatum in the dual cue condition ($p < 0.01$, two-tailed t-test).

Stimulation prolonged RTs significantly for most stimulation sites compared to control sessions (Fig.2B and C). RTs reflect the decision process including detection, recognition,

selection, and initiation of action. Although RTs were generally slowed, there was no increase of out-of-target errors ($p > 0.1$ in both monkeys) except for stimulation in the dIns (post-hoc Turkey's HSD test, $p = 0.002$ compared to the control session), showing that there was little movement impairment. Furthermore, the behavioral signs of anticipation, licking, and blinking, were largely unaltered in the stimulation sessions, indicating that the monkeys could discriminate between the CSs. These behavioral results showed that stimulation affected behavior in a context-dependent manner, with a reduction of approach behavior for stimulation during appetitive contexts and increased error responses in stimulation of the ventral part during aversive context. A comparison between the dorsal and ventral parts of the insula revealed that approach behavior was significantly disturbed by stimulation in the appetitive single-cue condition (two-way ANOVA with factors of cerebral regions and dorso-ventral gradient, $F_{2, 351} = 6.5$, $p = 0.002$ for regions, $F_{2, 351} = 7.42$, $p = 0.0068$ for dorso-ventral gradient, but not interaction effect, $p = 0.11$, and in the dual-cue condition (F2, 351 = 12.74, $p = 0.4 \times 10^{-4}$ for dorso-ventral gradient without interaction effect $p = 0.11$). A post-hoc Turkey's HSD test exhibited a significant difference between the dIns and the vIns in the appetitive single-cue condition ($p < 0.05$; Fig. 4B).

Figure 3 displays examples of stimulation sessions for each structure. These illustrate how the monkeys initialized approach behavior in the appetitive single-cue and dual-cue condition, but avoided aversive outcomes in the aversive single-cue condition in the control sessions (Fig. 3A). However, stimulation induced disadvantageous choices in the positive motivational context, as well as premature responses and non-initiated actions in the aversive single-cue condition for stimulation of the VP (Fig. 3B), the ventral striatum (Fig. 3C), and the ventral insula (Fig. 3D).

Stimulation in the dual-cue condition decreases appetitive approach behavior.

To understand the effect of stimulation on behavioral selection, we analyzed the dual-cue condition in which both appetitive and aversive CSs were presented simultaneously, which required monkeys to discriminate the CSs and to memorize their locations to obtain the most advantageous outcome. As shown above, without stimulation the monkeys selected approach behavior for the target associated with the appetitive outcome. In contrast, during stimulation sessions, the monkeys more frequently selected the target associated with the aversive outcome (Fig. 2D and E). The reconstruction map of sites leading to reduction of the appetitive approach inside the insula revealed that effective stimulation sites were concentrated in the middle to ventral parts (Fig. 4A). Only the most anterior part of the stimulated insula (AC+3) showed effective sites specifically in the ventral part. As for the striatum, disturbance effects were observed in the ventral part of the caudate nucleus and the putamen at the AC+3, as well as the central part of the vSTR at the level of AC+5. The posterior parts (AC0 to -2) that exhibited effective sites were distributed broadly from the dorsal to the ventral parts in the dual-cue condition (Fig. 4A). In the dual-cue condition, stimulation also increased both types of error responses: premature responses (Fig. 6A) and non-initiated actions (Fig. 5A). However, inside all studied structures, the number of sites that induced error responses was smaller than the number of sites with disturbance in behavioral selection. This result is illustrated in the figures for the striatum and insula (compare Fig. 5A and Fig. 6A to Fig. 4A), where fewer sites with induced errors were observed. Thus, in the dual-cue condition, the stimulation mainly disturbed value-based choice and facilitated disadvantageous choices rather than inducing error responses.

Context-dependent effects observed in the single-cue condition.

Even though appetitive and aversive CSs were presented simultaneously in the dual-cue condition, monkeys under stimulation more often chose to avoid the appetitive outcome and selected the aversive outcome (fig 2D and E), which is a disadvantageous choice. The fact that they reached for the target showed that they were able to maintain general task rules (e.g., no reaction during CSs presentation and waiting for the action target). We examined whether stimulation during CS encoding resulted either in impairment of decision-making processes (the value-based decision or the action selection) or facilitation of one of the options over the other (aversive avoidance over the appetitive approach). To test these conjectures, we used single-cue trials (where only one option was presented, either appetitive or aversive CS) that were included in the mixed sessions. The distribution of stimulation sites in the striatum eliciting reduction of approach behavior indicated a similar pattern as in the dual-cue condition (Fig. 4); however, the effective current intensity appeared different, especially in the caudate at the AC+3. The vIns at AC0 to -2 was less sensitive compared to those of the dual-cue condition (Fig. 4A and B). Thus, even when an appetitive CS was presented alone in the single-cue condition, the monkeys preferred to choose the target leading to a negative outcome (i.e., no outcome). One may therefore wonder whether avoidance behavior in the aversive single-cue condition may be facilitated by stimulation. However, avoidance behavior was generally significantly less often improved at any stimulation site, except for the striatum (binomial test, $p < 0.0001$ compared to negative effects). We found 26 stimulation sites (22% of effective stimulation sites) in the striatum where avoidance behavior was facilitated by the stimulation (Fig.4D-E). The average avoidance proportion for these sites was 78 ± 2% (mean ±SEM) in the aversive single-cue condition. These sites leading to avoidance facilitation were mainly found in the center of the striatum, mainly at AC+3 and +5 (Fig.4D). Furthermore, we found that these avoidance facilitation sites also increased avoidance behavior for appetitive outcome in the appetitive single-cue condition (Fig.4E).

Out of facilitation avoidance sites in the aversive single-cue condition, 16 (62%) showed increased avoidance in the appetitive single-cue condition. Of these, 11 (42% of all avoidance improvement) sites exhibited a reduction of appetitive choice in the dual-cue condition. Eight (31%) sites exhibited an improvement avoidance effect only, and the remaining two sites showed increased avoidance in the aversive single-cue condition and dual-cue condition. On average, avoidance behavior in the appetitive single-cue condition was 20 ± 2% (mean ± SEM), a significant increase compared to the control sessions ($t(231) = 11.167$, $p = 1.9 \times 10^{-23}$). Thus, the monkeys did not merely avoid the presented stimulus.

In order to investigate whether the reduction of approach behaviors was selective for either the single-cue or dual-cue condition, we compared the difference in appetitive approach behavior between the appetitive single-cue condition and the dual-cue condition. Only vIns stimulation led to a significant reduction of appetitive approach behavior in the dual-cue condition ($t(42) = 4.8364$, $p = 0.0002$), indicating that presentation of the aversive CS influences the monkey´s decision-making in the dual-cue condition. Thus, stimulation in most BG territories (the striatum and pallidum) showed disturbance of appetitive approach behavior to a similar degree between both appetitive contexts.

As for the error responses, we reconstructed the distribution of the sites inducing error responses in the single-cue condition (Figs. 5 and 6). The reconstruction maps inside the pallidum showed no topographic distribution of effective sites regarding non-initiated actions (Figs. 5) and premature responses (Fig. 6). The proportion of non-initiated action and premature response from the VP stimulation were comparable in the aversive single-cue-condition ($t(66) = 1.7919$, $p = 0.08$), but non-initiated actions were significantly more frequent in DP during the aversive single-cue condition ($t(68) = 2.957$, $p = 0.004$). Thus, GP stimulation induced both non-initiated actions and premature responses, but non-initiated actions were significantly increased by DP stimulation in a context-dependent manner. As for the striatum, although certain stimulation sites that induced error

responses were identified in the dorsal part, they were distributed close to the border between the dSTR and vSTR (Figs. 5 and 6). We found three main clusters for error response sites, one in the lateral part of the putamen, the second in the medial and ventral parts of the caudate nucleus at the AC+3, and a third cluster in the central part of the vSTR at the level of AC+5 (Figs. 5 and 6). The proportion of non-initiated actions in all of these striatal clusters during the aversive single-cue condition was significantly higher than the level of premature responses (pooled three clusters, t (438) = 5.1349, $p = 1.0 \times 10^{-7}$). Importantly, little behavioral effects were observed for stimulation of the internal capsule (IC), suggesting that the spreading electrical current in the IC was not responsible for the error responses induced by stimulation.

Stimulation in the aversive single-cue condition induced error responses in the ventral part of the insula (Figs.5C and 6C). The premature responses were observed more frequently in the posterior part (Fig. 6C, AC0 to -2) while non-initiated actions were mainly induced in the most anterior part, at AC+3 (Fig. 5C). Interestingly, we found that these induced non-initiated actions in the anterior and ventral parts of the insula were associated in monkey C with a decrease of licking during the outcome anticipatory period, resulting in non-significant differences among conditions (one-way ANOVA, F(2.39) = 1.53, $p = 0.229$). Although there was no significant difference between the number of premature responses and non-initiated actions inside the vIns as a whole (t (88) = 1.2767, $p = 0.21$, two-tailed t-test), stimulation of the posterior part of the vIns (AC0 to -2) in the aversive single-cue condition induced premature responses significantly more frequently (t (30) = 4.25, $p = 1.9 \times 10^{-4}$) than non-initiated actions. Thus, the distribution of premature response sites and non-initiated action sites were different inside the insula, with a partial overlap between both error types.

Differential latencies of premature responses suggest different functional perturbations.

Stimulation in the ventral part of all stimulated structures largely induced premature responses. The fact that premature responses were observed in a context-dependent fashion, for all studied structures, strongly contradicts the notion that the stimulation may have directly produced movements leading to the lever release before target presentation. Therefore, we hypothesize that these premature responses are aversive context-dependent escape behavior that reflect the functional implication of the stimulated territories. Indeed, by studying the latencies of these behavioral responses, we attempted to determine the (stimulation-disturbed) functional processing underlying the production of this error response. In order to do this, we measured the latency of premature responses elicited by stimulation of ventral structures in the aversive single-cue condition. We found that premature responses occurred with a mean latency that was longer than the end of stimulation in all ventral parts (Fig. 6D). When comparing latencies in the ventral regions, one-way ANOVA exhibited significant differences among stimulation sites ($F(2, 375) = 18.42$, $p = 2.34 \times 10^{-8}$). The fastest latency was found for stimulation of the VP (mean ± SEM, 0.85 ± 0.03 s, $p < 0.003$, post-hoc Turkey's HSD test compared with vSTR latency, 1.10 ± 0.03 s and the vIns, 1.16 ± 0.04 s). However, there was no significant difference between the corresponding latencies for vIns and the vSTR ($p = 0.8436$, post-hoc Turkey's HSD test). Moreover, analysis of the distribution of latencies between the three structures revealed different distributions for the three areas, and showed two main latency peaks: The VP and vSTR showed a first peak at 0.5 sec after stimulation onset, during stimulation and in reaction to the CS presentation on the screen. The ventral insula showed a peak at 1.1 to 1.2 sec after stimulation onset (Fig. 6D), which was thus 0.4-0.5 sec after stimulation offset during the first delay when the monkeys were preparing the selected action and evaluating the anticipated outcome (the expected reward and/or the aversive risk taking).

Discussion

To understand how cortico-BG circuits contribute to value-based choice and aversive-related behavior, we applied long-train microstimulation in three linked nuclei of these circuits while monkeys were performing an instrumental task. The stimulation was performed in the anterior part of the pallidum, STR, and insula during CS presentation. This focal and reversible approach has been highly effective for inducing context-dependent disturbances of choices and behaviors, with stronger effects in the ventral parts, especially in the VP and vSTR. Microstimulation induced biased decisions towards disadvantageous choice and resulted in reduction of appetitive approach-behavior in the dual-cue and appetitive single-cue conditions. In addition to these effects, two erratic behavioral reactions (non-initiated actions and premature responses) were more frequently induced in the aversive single-cue condition. Our results provide causal evidence that neuronal perturbation induced in the limbic territories of the VP and the vSTR - two BG nuclei well known to be involved in appetitive approach behavior – as well as the aIns also influences the selection of aversive behaviors (avoidance and escape behaviors) in negative contexts.

Microstimulation during CSs presentation is an effective tool to disturb value-based choice and behaviors.

Microstimulation allowed us to investigate disturbance effects of neuronal activity during the CS presentation on several sites and compare between three structures known to be organized in cortico-BG circuits. Previous studies have shown that continuous stimulation of the anterior dorsal part of the insula evoked ingestive behavior. Stimulation of the ventral portion of the insula induced disgust behavior expressed by rejection of food or vomiting (Catenoix et al., 2008; Caruana et al., 2011; Jezzini et al., 2012). Discontinuous long-train stimulation with the same parameters as used in the present study applied to

the lateral part of the vSTR (i.e., the ventral putamen) induced hypoactive states with loss of food motivation (Worbe et al., 2011). In this study, we restricted the stimulation to the CSs presentation period during which the monkeys made their decisions to approach or avoid the proposed choice(s). Stimulation during this CS encoding period was effective in inducing a negative bias in decisions and behaviors for all stimulated structures. In addition to these common effects, we found regional and territorial differences among the three studied structures. Importantly, internal capsula (IC) stimulation had no effect which strongly suggests that the effects observed at neighboring striatal sites are not due to activation of fibers passing through the structure but due to modifications of neuronal activity of striatal neurons.

One surprising results from stimulation of the striatum is that we did not find improved appetitive behavior, as observed previously during stimulation of the vSTR (Bichot, Heard and Desimone, 2011) and Caudate nucleus (Williams and Eskandar, 2006). This discrepancy with previous results may reflect differences in the stimulation period that we chose (CS presentation versus outcome delivery) and the context (appetitive-aversive) of the stimulation. In the previous studies, stimulation was applied during the outcome period and only in an appetitive context during reward learning.

Motivation context influences the behavioral expression of stimulation disturbances in the ventral cortico-BG circuit.

Stimulation induced heterogeneous behavioral effects, increase of aversive behaviors in the negative context, and reduction of appetitive approach behavior in the positive context. In other words, the motivational context appeared to be a crucial determinant for the behavioral expression of stimulation-induced functional disturbance. The present results confirmed that CS-encoding is crucial for appropriate decision-making under learned appetitive-aversive contexts. Importantly, behavioral markers of outcome anticipation -

i.e., licking and blinking for positive and negative value expectation, respectively - were largely maintained during the stimulation sessions. Furthermore, the monkeys preserved the general ability to perform tasks during stimulation (i.e., reaching to the target). Nevertheless, stimulation induced heterogeneous behavioral effects in a context-dependent manner, suggesting that monkeys could discriminate the motivational context by CSs. The fact that this ability was preserved may reflect that the duration of the stimulation in our study was shorter than the duration of CSs presentation in Amemori and Graybiel (2012). The remaining 300 ms of the presentation period without stimulation may be enough to recognize the motivational context of the trial. This raises the question of how electrical stimulation may have affected neuronal activity. Did it disrupt neuronal activity or did it activate a subgroup of neurons to the disadvantage of another subgroup? Although it is very difficult to answer this question (as discussed previously (Worbe et al., 2011), previous neuronal recordings can provide more information on the context-dependent effects of stimulation. Disturbance of positive value encoding during CS presentation may indirectly generate uncertainty about the expected outcome and thus induce delayed RTs, as observed for most stimulation sites (see Fig. 2). Furthermore, an increase of premature responses or non-initiated actions was observed in the aversive context. We previously identified neurons preferring aversive motivational context in the VP (Saga et al., 2017) and the striatum (Richard et al. 2014). Some of them expressed anticipatory activity preceding aversive events (CS or outcome) or during a period of aversive uncertainty (Saga et al., 2017). As previously shown with bicuculline injections, an increase of these activities can lead to an excess of negative contextual information (Saga et al., 2017). Thus, the escape behavior observed here may reflect a sustained increase in the activity of aversive anticipatory neurons, which has already been observed during aversive anticipation (Saga et al., 2017). Finally, activation of these aversive

neurons during the CS values encoding can bias the decisions towards not initiating appetitive approach.

Specific functional involvement of different sub-territories in the cortico-BG network

The most prominent differences between stimulated sub-territories concerned sensitivity to stimulation intensity and the latency of premature response. Whereas the current intensity required in the GP was small (Table 1), the highest current intensity was required in the Insula. Moreover, error responses were more pronounced during stimulation of VP and vSTR, suggesting that limbic BG territories may be crucial nodes in this brain network involved in value-based motivated behaviors. Perhaps unexpectedly, the VP showed the fastest latency of premature response, with a phasic peak during stimulation. The vSTR also exhibited its peak latency during stimulation, but the latencies were distributed broadly, with an intermediate mean latency between VP and vIns. These delayed components in the vSTR or anterior-ventral insula were initiated during the delay period, around 300ms after the stimulation offset (see Fig.6D). These differential latencies may reflect different neural processing as well as different properties or anatomical connections among these territories. The ventral caudate nucleus receives inputs preferentially from the orbitofrontal cortex (OFC) and the ACC, two areas of the ventro-medial Prefrontal Cortex (VMPFC) (Kunishio and Haber, 1994; Haber et al., 1995; Ongur and Price, 2000; Haber et al., 2006; Calzavara et al., 2007), well known to be involved in value-based decision-making processes. The OFC plays a major role in expected value computation from CS information (Tremblay and Schultz, 1999; Gottfried et al., 2002; Padoa-Schioppa and Assad, 2006; Klein-Flugge et al., 2013) and the pregenual ACC contributes to the control of approach-avoidance conflict behavior (Amemori and Graybiel, 2012). As in the VMPFC, value-representing neuronal activity during anticipation and response to the cue has also been observed in the VP (Tachibana and Hikosaka, 2012; Saga

et al., 2017) and the anterior caudate nucleus (Hollerman et al., 1998). Indeed, the caudate and ventral STR encode different discounting values in intertemporal choice (Cai et al., 2011). The differential latencies of premature responses suggest that the VP may be a crucial command node in the network. Our recording in the insula exhibited very few neurons encoding motivational CS values (unpublished data). Instead, the majority of neurons inside the aIns mainly encoded the outcome. There were topographical distributions of neurons preferring the aversive outcome in the ventral part, and both appetitive and aversive outcomes were intermixed in the dorsal part. These results are consistent with the stimulation result that premature responses were observed more in the ventral region (see Fig. 6) and may reflect why latencies of premature responses were longer than those of VP. A meta-analysis suggests that the insula is not directly involved in the evaluation of subjective CS values but rather in the anticipation and evaluation of outcomes, especially for negative outcomes (Bartra et al., 2013). Previously, activity of the aIns was found to be modulated during anxiety anticipation (Liotti et al., 2000; Simmons et al., 2006; Yang et al., 2012; Engelmann et al., 2015), unpleasant feeling (Corradi-Dell'Acqua et al., 2016), and disgust (Calder et al., 2007). Given these findings, stimulation of the ventral part may urge monkeys to escape from the aversive context due to a negative change in motivational state.

Another noteworthy differential effect we observed was the increase in avoidance behavior produced by stimulation of the central part of the vSTR (see Fig. 4D). For some of these stimulation sites, we even induced avoidance of appetitive outcomes during the appetitive single-cue condition (62%), suggesting that these sites may be a 'pure' avoidance induction area. It is interesting to note that this region corresponds to the territory of the vSTR that receives projections from the ACC in which Amemori and Graybiel (2012) induced avoidance behaviors by stimulation in monkeys. In humans, the central part of the vSTR

is activated in learned active avoidance (Jensen et al., 2003; Delgado et al., 2009) and has shown structural anomalies in obsessive compulsive disorders and anxiety disorders (Radua et al., 2010). This is also the same striatal territory where we previously induced compulsive grooming and stereotypical behavior (biting fingers) usually induced by stress (Worbe et al. 2009; Sgambato-Faure et al. 2013).

Taken together, these stimulation effects in the studied structures, especially inside the ventral striatum, suggest that different sub-territories in the cortico-BG limbic circuits play different crucial roles in value-based decision-making and the control of approach-avoidance behaviors.

Conflict of interest

None declared.

Data accessibility

Data are available from the corresponding author upon request.

Author contributions

YS, and LT designed the research. LT and CR supplied the funding; YS performed the experiments. YS analyzed data under the supervision of LT. YS prepared figures. All authors wrote the first draft of the manuscript and revised and approved the final version of the manuscript.

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Abbreviations

Anterior cingulate cortex (ACC), anterior commissure (AC), anterior Insula (aIns), appetitive (AP), aversive (AV), Basal ganglia (BG), conditioned stimulus (CS), dorsal Insula (dIns)dorsal striatum (dSTR), globus pallidus (GP), internal capsula (IC), magnetic resonance imaging (MRI), movement time (MT), orbitofrontal cortex (OFC), reaction time (RT), striatum (STR), unconditioned stimuli (US), ventral pallidum (VP), ventral striatum (vSTR), ventro-medial Prefrontal Cortex (VMPFC)

Figure legends

Figure.1

Behavioral tasks, behavioral results in the control condition, and division of structures. **A**, Dual-cue condition in which two different CSs were presented in a trial. **B**, Single-cue condition in which monkeys performed an appetitive single-cue trial (upper) and an aversive single-cue trial (bottom). Yellow zone indicates stimulation period (0.7s). **C**, Performance in control sham sessions. The proportion of complete trials was indicated for each monkey, T and C, in each trial. The proportion of approaches in the appetitive single-cue trial, dual-cue trial, and avoidance behavior in the aversive single-cue trials are indicated in the middle panel. The right panel indicates the proportion of error response trials. **D**, Licking and blinking behaviors measured 0.5s before delivery of outcome. **E**, Division of the pallidum. Red line divides the pallidum into dorsal and ventral at the level of AC (AC+0) and the middle of pallidum (AC-1). **F**, Division of the STR. Red lines divide dorsal and ventral parts at the level of AC+5 and AC+3. **G**, Division of the insula at the level of AC +3 to AC-2. Dual; dual-cue condition, AV; aversive single-cue condition. AP; appetitive single-cue condition.

Figure.2

The proportion of error response, changes of reaction times (RTs) and the proportion of avoidance choices in control sham sessions and stimulation sessions. **A**, The proportion of error responses (mean ± SEM) in the dual-cue condition (black colored circles), the appetitive single-cue condition (blue colored circles), and the aversive single-cue condition (red colored circles). Bars indicate mean ± SEM of RTs in Monkey T (B) and C (C). RTs in dual-cue (circles), appetitive single-cue condition (blue colored circles), and aversive single-cue condition (red colored circles). The proportion of avoidance choices in each condition

(D: Monkey T, E: Monkey C). In the dual-cue condition, this reflects the proportion of choices avoiding the appetitive outcome. Circles and bar indicate mean ± SEM. Cont: control sham session, dIns: dorsal insula, vIns: ventral insula, dSTR: dorsal part of the STR, vSTR: ventral part of STR, DP: dorsal pallidum, VP:ventral pallidum. The star mark indicates in each panel significance by performing two-tailed t-test compared with RTs in control; * $p < 0.05$, ** $p < 0.03$, *** $p < 0.01$.

Figure.3

Examples of behavioral consequences in sham control and stimulation sessions. **A**, An example of performance in the sham control stimulation session. The number along the horizontal axis indicates the number of trials. Marks represent behavioral outcome in each trial. Behavioral consequence of stimulation in the VP with 0.4 mA (**B**), that of the vSTR with 0.6 mA (**C**), and that of vIns with 0.4 mA (**D**). AP context: trials in the appetitive single-cue condition, AV context: trials in the aversive single-cue condition, and dual-cue: trials in the dual-cue condition. Each mark indicated in the rectangle corresponds to the following. Green dot: approach, red dot: avoidance, blue dot: non-initiated action, red multiple marks: premature response occurring in pre-CS period, black multiple marks: premature response in the peri-CS period, black cross: out of the target.

Figure.4

Distribution of sites induced disadvantageous choice and improvement of avoidance behavior. **A**, Decreased approach of the target associated with appetitive outcomes due to stimulation of the pallidum (left), STR (middle), and insula (right). Dot size represents effective current intensity. Small horizontal (Monkey T) or vertical (Monkey C) lines

represent no significant effect. **B**, Decrease of approach behavior in the appetitive single-cue condition. **C**, Decrease of avoidance behavior in the aversive single-cue condition. D, the distribution of avoidance improvement sites in the vSTR. Scale bar indicates 1mm. E, the proportion of avoidance between the aversive single-cue condition (horizontal line) and that of appetitive single-cue condition (vertical line). Gray dashed line and zone indicate mean ± 2SD in the control sham sessions. Solid line and zone indicate mean ± 2SD of the stimulation session in each single-cue condition. A dot represents the proportion of avoidance in a single stimulation session.

Figure.5

Distribution of the proportion of non-initiated actions. The distribution of non-initiated actions in the dual-cue condition (**A**), appetitive single-cue condition (**B**), and the aversive single-cue condition (**C**). Color and size of marks indicate proportion of premature response and current intensity. Small horizontal (Monkey T) or vertical (Monkey C) lines represent no significant effect.

Figure.6

Distribution of stimulation sites that induced premature response. **A**, Premature response induced in the dual-cue condition. Premature response in the appetitive (**B**) and aversive single-cue conditions (**C**). The figure format is the same as in figure.5. **D**, The latency of premature responses in each stimulated ventral structure. The latency is represented in the histogram aligned on CS onset in the VP, STR, and insula. Gray zone indicates stimulation duration (0.7s).

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Table 1: The number of negative, positive effects in the single-cue condition, and effective current intensity.

Both monkeys	dSTR *n* (%)	vSTR	DP	VP	dIns	vIns
Stimulation sites	74	186	40	47	47	48
Effective sites	37(50)	116(62)	28(70)	22(47)	21(45)	32(67)
Effect on error response	9(24)	30(26)	6(21)	2(9)	0(0)	9(28)
Effect on approach	21(56)	45(39)	18(64)	2(9)	15(83)	19(59)
Both	7(19)	40(34)	4(14)	18(82)	5(24)	8(25)
Improvement of avoidance in aversive single*	1(3)	20(17)	0(0)	3(14)	2(9)	3(9)
Average intensity (mA)	0.49±0.13	0.41±.016	0.25±0.15	0.19±.011	0.72±0.13	0.62±0.11
Chi-square test (Dorsal vs Ventral)	P=0.274		P=0.955		P=0.008	

*Effects could contain both negative effect and improvement of avoidance.

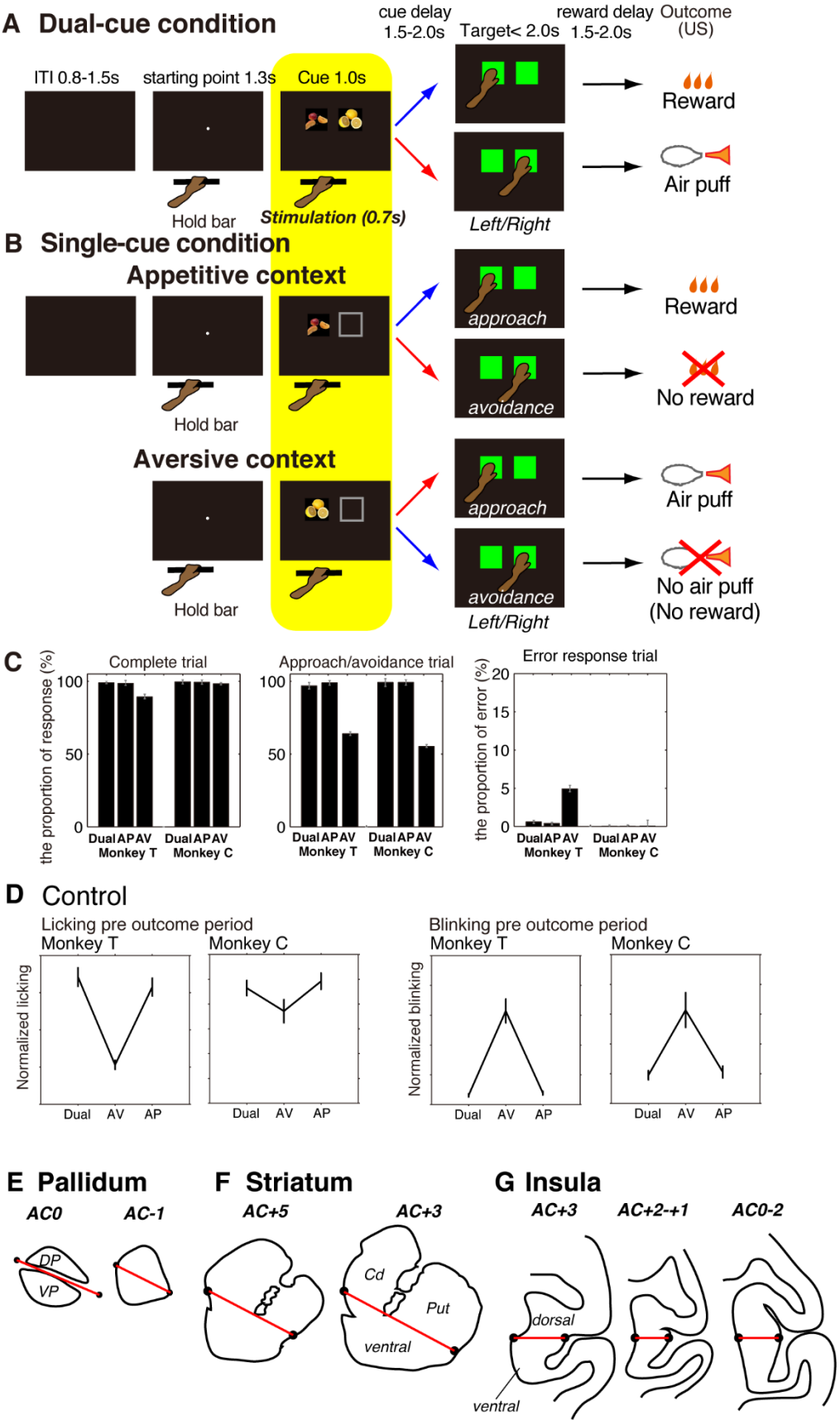
A Dual-cue condition
cue delay 1.5-2.0s
Target< 2.0s
reward delay 1.5-2.0s
Outcome (US)
ITI 0.8-1.5s
starting point 1.3s
Cue 1.0s
Hold bar
Stimulation (0.7s)
Left/Right
Reward
Air puff
B Single-cue condition
Appetitive context
approach
avoidance
Reward
No reward
Hold bar
Aversive context
approach
avoidance
Air puff
No air puff (No reward)
Hold bar
Left/Right
C
Complete trial
Approach/avoidance trial
Error response trial
the proportion of response (%)
the proportion of error (%)
100
50
0
20
15
10
5
Dual AP AV
Monkey T
Monkey C
D Control
Licking pre outcome period
Blinking pre outcome period
Monkey T
Monkey C
Normalized licking
Normalized blinking
Dual AV AP
E Pallidum
AC0
AC-1
DP
VP
F Striatum
AC+5
AC+3
Cd
Put
ventral
G Insula
AC+3
AC+2-+1
AC0-2
dorsal
ventral

Figure 1

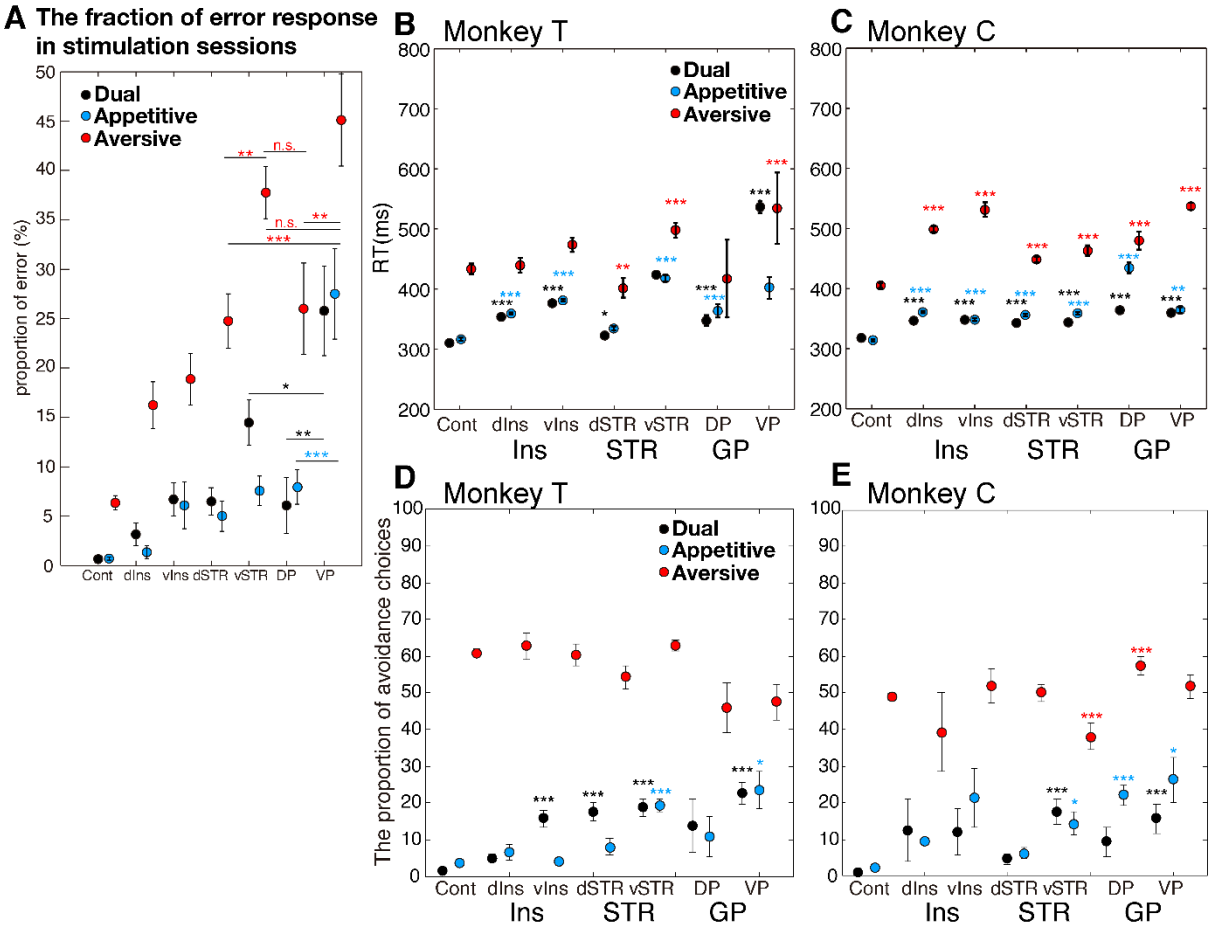
A
The fraction of error response in stimulation sessions
Dual
Appetitive
Aversive
proportion of error (%)
n.s.
Cont dIns vIns dSTR vSTR DP VP
B
Monkey T
RT(ms)
C
Monkey C
Ins
STR
GP
D
Monkey T
The proportion of avoidance choices
E
Monkey C

Figure 2

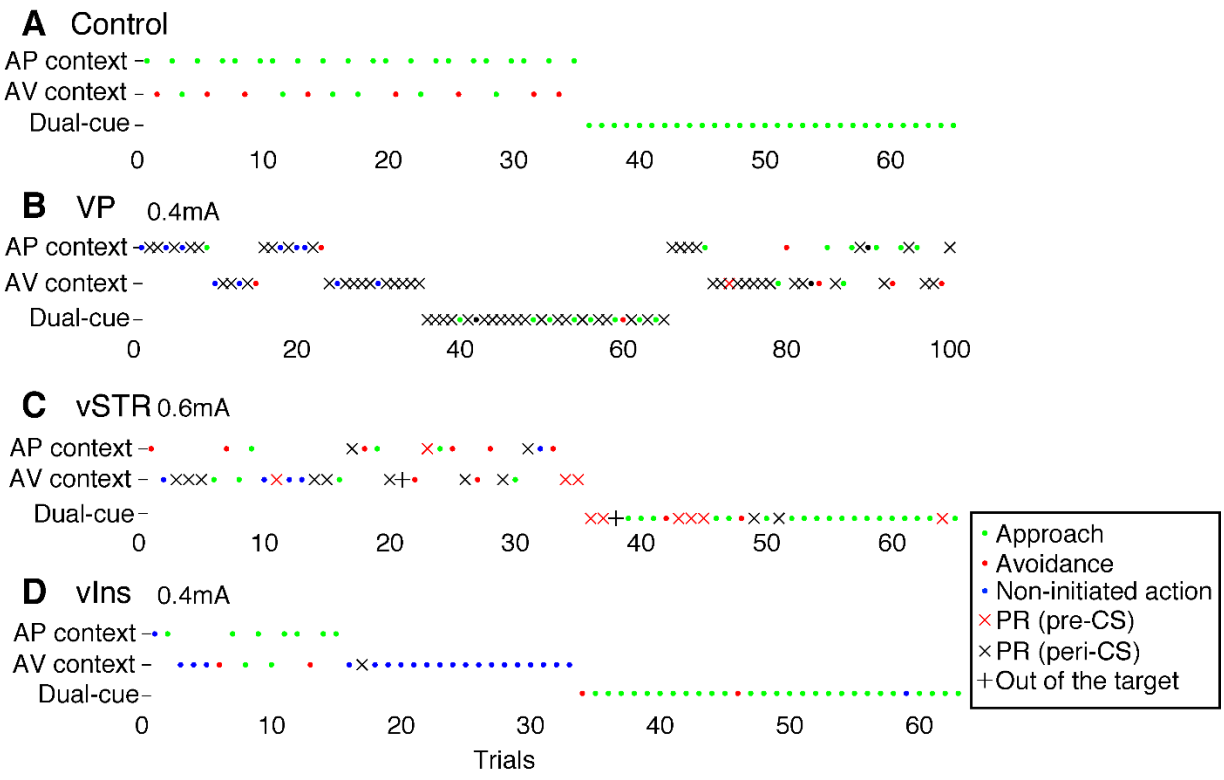
A Control
AP context
AV context
Dual-cue
0
10
20
30
40
50
60
B VP 0.4mA
AP context
AV context
Dual-cue
0
20
40
60
80
100
C vSTR 0.6mA
AP context
AV context
Dual-cue
0
10
20
30
40
50
60
D vIns 0.4mA
AP context
AV context
Dual-cue
0
10
20
30
40
50
60
Trials
Approach
Avoidance
Non-initiated action
PR (pre-CS)
PR (peri-CS)
Out of the target

Figure 3

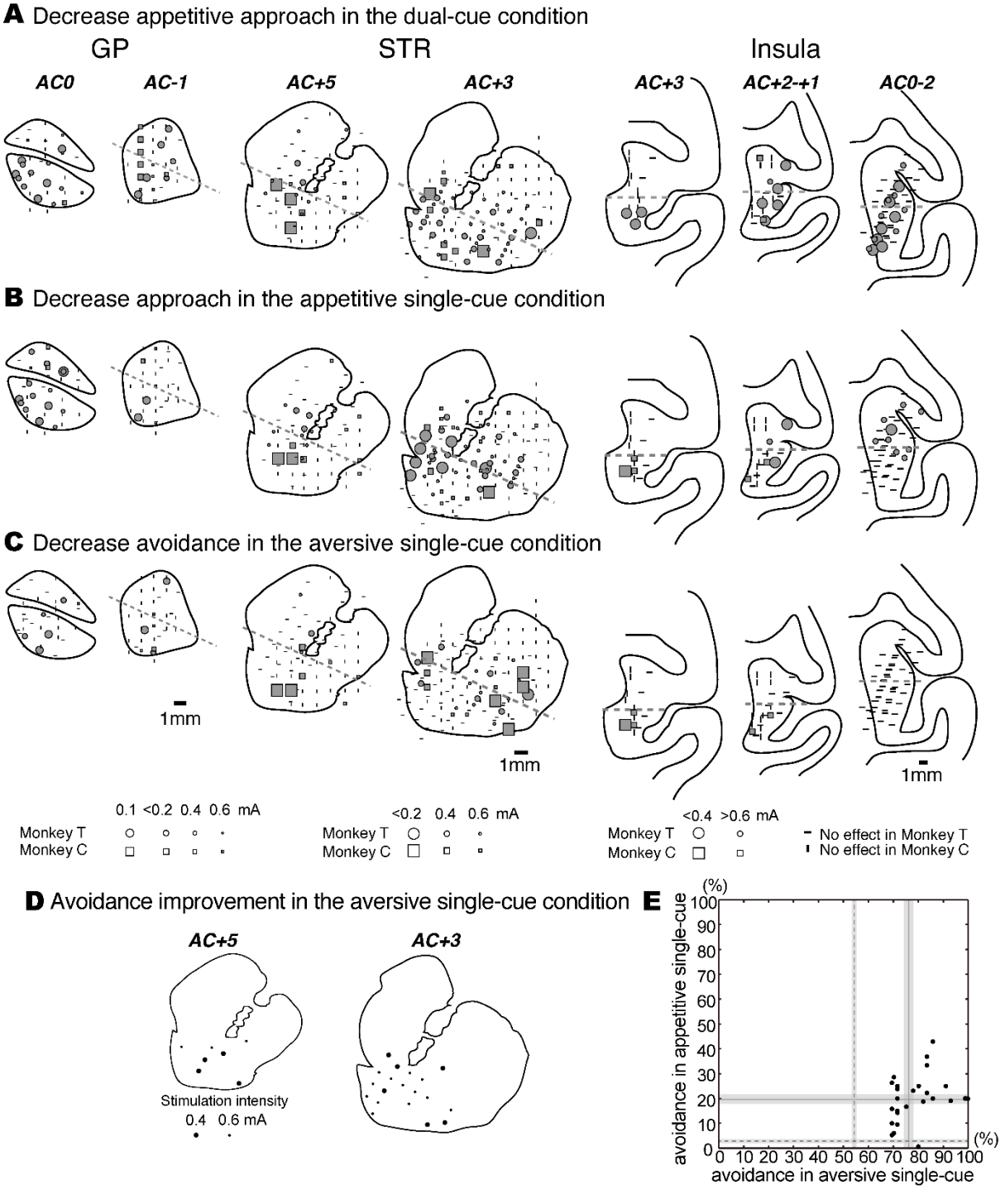
A Decrease appetitive approach in the dual-cue condition
GP
STR
Insula
AC0
AC-1
AC+5
AC+3
AC+3
AC+2-+1
AC0-2
B Decrease approach in the appetitive single-cue condition
C Decrease avoidance in the aversive single-cue condition
1mm
1mm
1mm
0.1 <0.2 0.4 0.6 mA
Monkey T
Monkey C
<0.2 0.4 0.6 mA
Monkey T
Monkey C
<0.4 >0.6 mA
Monkey T
Monkey C
No effect in Monkey T
No effect in Monkey C
D Avoidance improvement in the aversive single-cue condition
AC+5
AC+3
Stimulation intensity
0.4 0.6 mA
E
(%)
avoidance in appetitive single-cue
100
90
80
70
60
50
40
30
20
10
0
0 10 20 30 40 50 60 70 80 90 100
(%)
avoidance in aversive single-cue

Figure 4

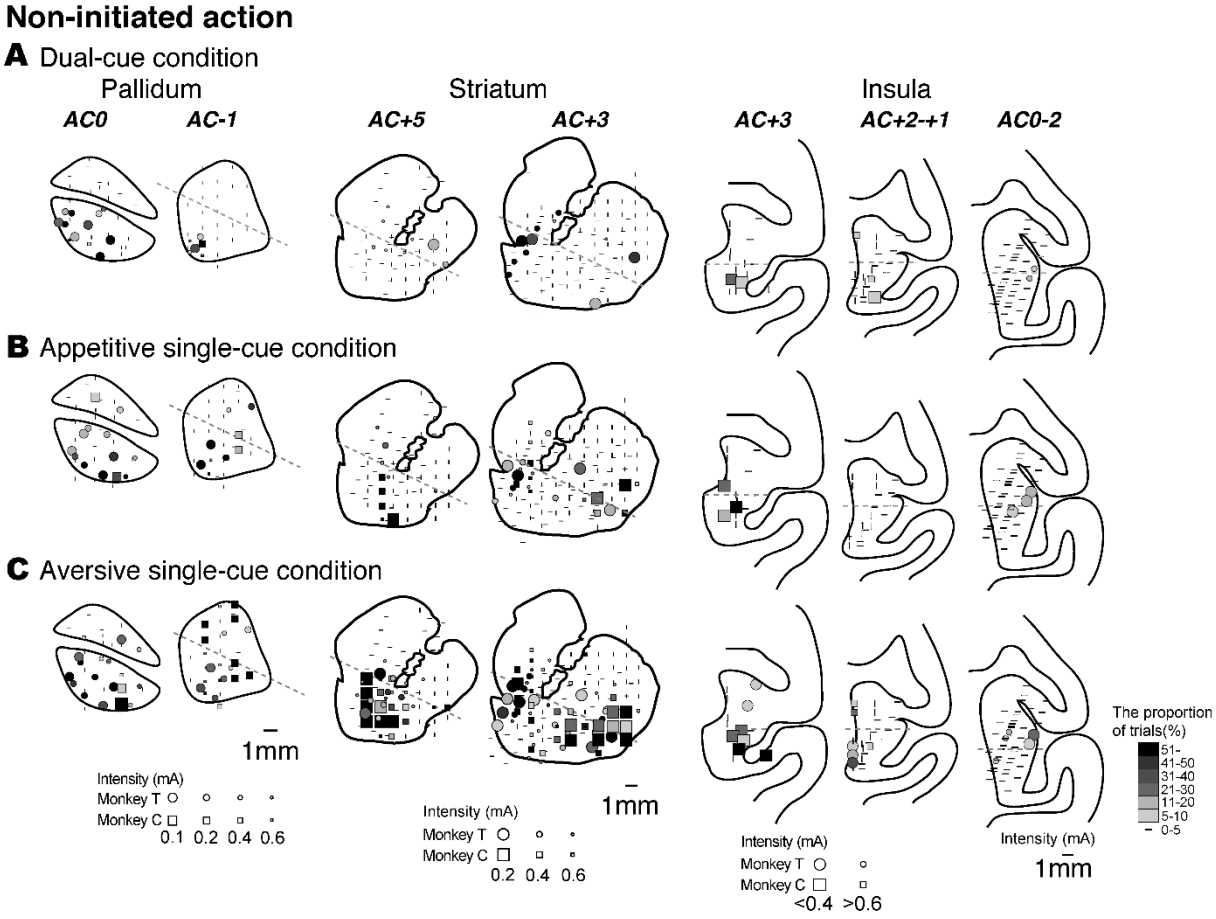
Non-initiated action
A Dual-cue condition
Pallidum
Striatum
Insula
AC0
AC-1
AC+5
AC+3
AC+3
AC+2-+1
AC0-2
B Appetitive single-cue condition
C Aversive single-cue condition
1mm
Intensity (mA)
Monkey T
Monkey C
0.1 0.2 0.4 0.6
0.2 0.4 0.6
<0.4 >0.6
The proportion of trials(%)
51-
41-50
31-40
21-30
11-20
5-10
0-5

Figure 5

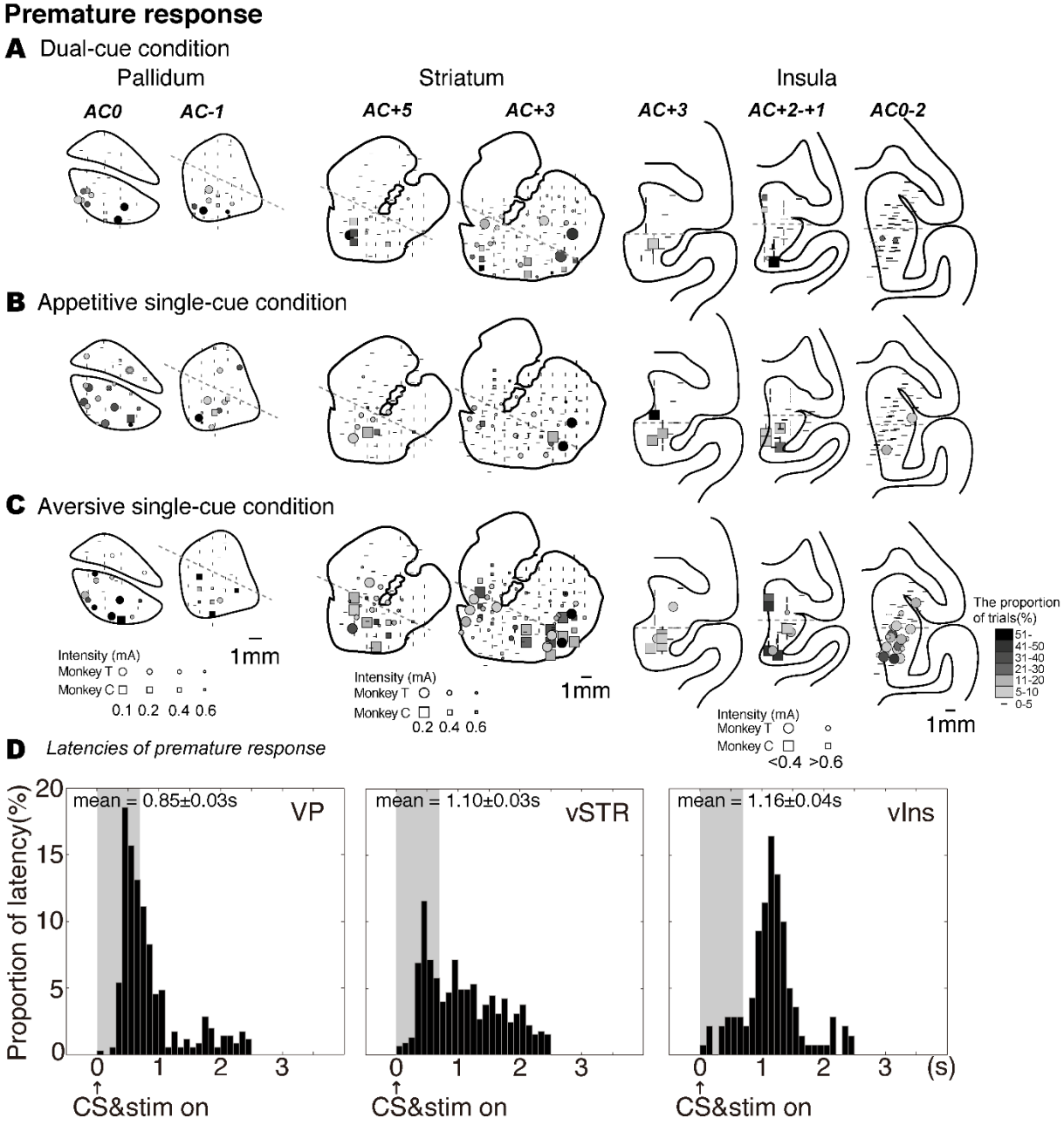
Premature response
A Dual-cue condition
Pallidum
Striatum
Insula
AC0
AC-1
AC+5
AC+3
AC+3
AC+2-+1
AC0-2
B Appetitive single-cue condition
C Aversive single-cue condition
Intensity (mA)
Monkey T
Monkey C
0.1 0.2 0.4 0.6
1mm
0.2 0.4 0.6
<0.4 >0.6
The proportion of trials(%)
51-
41-50
31-40
21-30
11-20
5-10
0-5
D Latencies of premature response
Proportion of latency(%)
mean = 0.85±0.03s
VP
mean = 1.10±0.03s
vSTR
mean = 1.16±0.04s
vIns
CS&stim on
(s)

Figure 6